



Research paper

An operant-based detection method for inferring tinnitus in mice



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HIGHLIGHTS

- A sound-based avoidance detection (SBAD) method was developed for detecting tinnitus in mice.
- Increases in error rates during the SBAD No-Go task were strongly correlated with the presence of tinnitus.
- The SBAD method was validated by two additional functional assays.
- The SBAD method can be used to detect both acute and chronic tinnitus in mice.

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ABSTRACT

Background: Subjective tinnitus is a hearing disorder in which a person perceives sound when no external sound is present. It can be acute or chronic. Because our current understanding of its pathology is incomplete, no effective cures have yet been established. Mouse models are useful for studying the pathophysiology of tinnitus as well as for developing therapeutic treatments.

New method: We have developed a new method for determining acute and chronic tinnitus in mice, called sound-based avoidance detection (SBAD). The SBAD method utilizes one paradigm to detect tinnitus and another paradigm to monitor possible confounding factors, such as motor impairment, loss of motivation, and deficits in learning and memory.

Results: The SBAD method has succeeded in monitoring both acute and chronic tinnitus in mice. Its detection ability is further validated by functional studies demonstrating an abnormal increase in neuronal activity in the inferior colliculus of mice that had previously been identified as having tinnitus by the SBAD method.

Comparison with existing methods: The SBAD method provides a new means by which investigators can detect tinnitus in a single mouse accurately and with more control over potential confounding factors than existing methods.

Conclusion: This work establishes a new behavioral method for detecting tinnitus in mice. The detection outcome is consistent with functional validation. One key advantage of mouse models is they provide researchers the opportunity to utilize an extensive array of genetic tools. This new method could lead to a deeper understanding of the molecular pathways underlying tinnitus pathology.

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1. Introduction

Subjective tinnitus is the perception of sound, such as ringing, roaring, or clicking in the ears, without an acoustic stimulus. It affects approximately 10–15% of American adults (Tunkel et al.,

2014), roughly 1–5% of whom experience levels that are severe or disabling (Cooper, 1994; Ryan and Bauer, 2016). Tinnitus is the most commonly reported service-related disability for veterans returning from recent conflicts (Veteran Benefits Administration, 2013). People with tinnitus often report significant morbidities, including sleep deprivation and emotional complications (Folmer and Griest, 2000), and they are also at increased risk for depression and anxiety disorders (Crocetti et al., 2009; Hébert et al., 2012; Shargorodsky et al., 2010). Currently, there are no approved drugs capable of preventing or treating tinnitus, partly due to our incomplete under-

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standing of its underlying molecular mechanisms. Mouse models, which provide extensive genetic information and well-established tools for genetic manipulation, could be useful for overcoming this obstacle.

Behavioral tests based on operant conditioning (OC) have been established to detect tinnitus in animals (for recent review, Brozoski and Bauer, 2016; Hayes et al., 2014; Lobarinas et al., 2008), and most of these are based on water-licking behavior in rats (Bauer and Brozoski, 2001; Heffner and Harrington, 2002; Jastreboff et al., 1988a, 1988b; Jones and May, 2016; Lobarinas et al., 2004; Pace et al., 2016; Ruttiger et al., 2003). Alternative OC-based methods have also been developed (Heeringa et al., 2014; Sederholm and Swedberg, 2013; Stolzberg et al., 2013; Yang et al., 2011; Zhang et al., 2014; Zheng et al., 2010). However, few of these methods are established for mice. In addition, most are time-consuming and lack the capability for monitoring possible confounding factors (Turner et al., 2006). Currently, the most widely used test for detecting tinnitus-like behavior is an alternative to OC-based methods based on gap prepulse inhibition of the acoustic startle reflex (GPIAS) (Turner et al., 2006, 2012). Under normal conditions, the startle reflex elicited by a sudden loud sound is reduced when the stimulus is preceded by a silent gap embedded in background noise or a tone. The underlying assumption of GPIAS is that, in subjects with tinnitus, the startle response should be more pronounced since the tinnitus “fills” (Turner et al., 2006), or partially fills, the silent gap (Turner et al., 2012). The GPIAS ratio, which is calculated by dividing the startle amplitude generated during trials containing the brief silent gap by the amplitude of the startle response in trials with no gaps, is higher in subjects that demonstrate significantly reduced gap inhibition, such as those with tinnitus. GPIAS has been modified for application to mice (Longenecker et al., 2011, 2012, 2014; Grimsley et al., 2015; Middleton et al., 2011). The premise upon which GPIAS is based—that tinnitus makes it difficult to perceive a silent gap—has been challenged by both animal (Hickox and Liberman, 2014; Radziwon et al., 2015) and human studies (Campolo et al., 2013; Fournier and Hebert, 2013; Boyen et al., 2015). Thus, new OC-based methods for detecting tinnitus in mice would be helpful to cross-validate the GPIAS method.

Here, we describe a new OC-based method, named sound-based avoidance detection (SBAD), which employs negative reinforcement (Estes and Skinner, 1941; Schleidt and Kickert-Magg, 1979) as a means of detecting tinnitus in mice. One major advantage of this new method is its “Go” paradigm, which monitors possible confounding factors, such as learning and motivation, thus reducing detection errors. A second paradigm, “No-Go,” demonstrates that the SBAD method distinguishes between animals that develop tinnitus from those that do not. In addition, we validated our observed behavioral findings with two congruent functional assays for cochlea and midbrain activities, further confirming SBAD as an effective method in the detection of tinnitus in mice.

2. Materials and methods

2.1. Animals

C57BL/6J mice (<https://www.jax.org/strain/000664>) were obtained from Jackson Laboratory (Bar Harbor, ME). Because early age-related hearing loss has been well observed in C57BL/6J mice (Henry and Chole, 1980), all behavior tests were performed when mice were between two and four months of age to avoid possible inconsistencies for tinnitus induction due to age-related hearing loss. Four mice (the same gender) from different groups were randomly housed in each cage under a 12:12 h light-dark cycle (lights on at 8:00 a.m.) and constant temperature (25 °C). Procedures used in this study were approved by the Institutional

Animal Care and Use Committees at Washington University, St. Louis, MO, and Northeast Ohio Medical University, Rootstown, OH.

2.2. SBAD method

Previous studies have demonstrated that Go/No-Go OC procedures provide reliable measures of auditory sensitivity in mice (Prosen et al., 2003; Klink et al., 2006; Radziwon et al., 2015). We used both salicylate- and noise-induced tinnitus models to develop the SBAD method, operating under the premise that salicylate would produce an acute, temporary form of tinnitus in mice, while noise would produce a chronic form.

2.2.1. Apparatus

The shuttle box hardware was specially built for the SBAD method by Kinder Scientific (Poway, CA), based on the Hamilton Kinder LM1000 system. The apparatus consisted of two compartments of equal size (12.5 cm × 20 cm × 20 cm) connected by a programmable shutter (Fig. 1). Mouse movement between compartments was measured via light-emitting diode (LED) beams present in each chamber. Sound cues were delivered via piezo tweeters located in the ceiling of each compartment approximately 20 cm above each mouse. The sound cue was randomly played with white noise or narrow-band noise (NBN, 1/3 octave, 6 dB/octave roll-off) centered at 8, 10, 12.5, 16, and 20 kHz, and the sound intensity was randomly varied at 75, 80, or 85 dB sound pressure level (SPL). The entire system including data acquisition was controlled by tinnitus avoidance software developed by Kinder Scientific, which was run on a PC operating in Windows XP.

2.2.2. Training and testing procedures

Training – We devised a Go trial to monitor motor, motivation, and learning/memory functions, and a No-Go trial to monitor tinnitus-like behavior (Fig. 1). Both trials were randomly assigned at each training and testing session. Training sessions started with a 2 min acclimation period followed by 100 trials per day for a total of 30–40 min per animal. For the Go trials, animals were trained to move from one compartment to another upon presentation of any sound. As soon as they moved to the other compartment, the shutter would be closed. If they did not move through the shutter, they received a shock 5 s after the start of sound stimulation. For the No-Go trials, animals were trained to stay in the compartment when no sound was delivered. If they moved through the shutter within 10 s, they received a shock but the shutter would remain open. The shock current strength was adjusted based on the mouse's body weight (15–28 g) from 0.1 mA to 0.4 mA. The shock was turned off either after the mouse moved to the other chamber or at the end of the trial for both Go and No-Go scenarios. Sound stimuli were presented randomly, both in frequency and intensity. The inter-trial interval (ITI) was fixed at 4 s so that multiple testing units in the system could be synchronized with the Kinder Scientific software (Poway, CA). It took 10–15 days for all mice to achieve a stable level of performance (above 90% correct).

Testing – During testing sessions, negative reinforcement (electrical shock at 0.4 mA) continued being applied after errors in Go trials, and the shutter would be closed as soon as the animal moved to the other compartment. However, in No-Go trials, a shock was no longer administered and the shutter would be closed right away if an animal crossed into the other compartment. Such actions—moving from one compartment to another in the absence of an external sound stimulus—were used as an indication of tinnitus. If a shock had been given after an animal with tinnitus crossed during a No-Go trial, the animal would extinguish this type of “error” behavior quickly, a phenomenon we had observed in preliminary studies. When tinnitus was induced by salicylate injection, animals were tested for five consecutive days because we observed

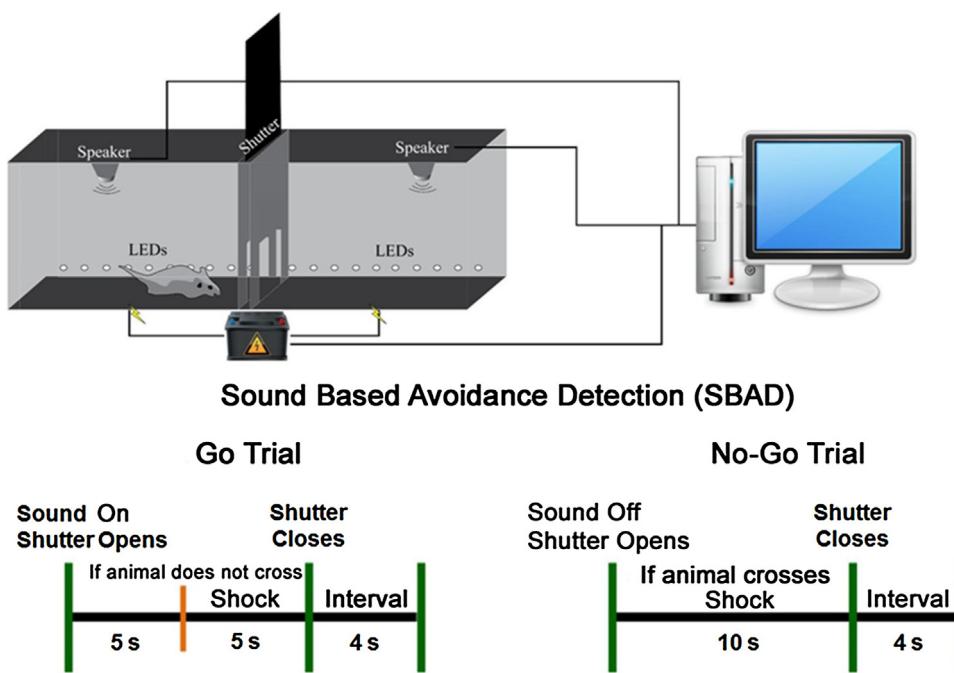


Fig. 1. Schematic representation of the SBAD method using the Go/No-Go paradigm.

Shuttle-box apparatus to detect mouse movement between two chambers connected by a programmable shutter. Sound was delivered via piezo tweeters in the ceiling of the chamber that contains the mouse. Light from light-emitting diodes (LED) detected mouse movement between the chambers. The Go/No-Go paradigm. The Go trial: Mouse moved between chambers in response to sound. Failure of movement after the initial 5 s resulted in deliverance of an electrical shock ("Shock") to force moving to the other chamber. The No-Go trial: In the absence of sound, mouse movement between chambers at any time within 10 s resulted in the deliverance of a shock, which forced the animal to stop moving between chambers. Shutter closed after 10 s.

in our preliminary experiments that the appearance of tinnitus-like behavior in each animal became more consistent following several salicylate injections. When tinnitus induction occurred following noise exposure, animals were tested at 2 weeks, 1 month, and 2 months after noise exposure.

In order to repeatedly detect tinnitus-like behavior following noise exposure, we addressed the problem of extinction—the process by which a conditioned behavior gradually dies out after the removal of reinforcement—with two major modifications. First, we designed the No-Go trial to indicate tinnitus-like behavior based on a mouse's natural instinct to remain in the same location. Second, during testing sessions of the No-Go trial, the shuttle was programmed to close as soon as the animal moved to the opposite chamber, so that mice with tinnitus would not be aware of their mistake when they moved to the other side.

2.3. Tinnitus induction

2.3.1. Salicylate

SBAD training began for each animal at 6 weeks of age. Based on our preliminary experiments, mice were then given intraperitoneal (i.p.) injections of sodium salicylate (350 mg/kg, Sigma). Injections were administered one hour before testing and at approximately the same time each day.

2.3.2. Noise exposure

After mice achieved stable performance during SBAD training, they received unilateral noise-induced trauma. Before noise exposure, mice were anesthetized with an injection of a ketamine/xylazine mixture (i.p., 100/10 mg/kg). One ear was blocked with a sound-reducing compound (Decidamp 2 Ear Plugs, Fisher Scientific, Pittsburgh, PA), held in place with stomahesive (ConvaTec, Greensboro, NC). The next day, fully awake mice were placed in a sound-attenuating chamber and exposed to 120 dB

broadband noise (4–25 kHz) for 2 h. The noise was presented using an amplifier (Cdi 100, Crown Audio, Elkhart, IN) connected to a Selenium D3500 Ti-ND speaker (JBL, Northridge, CA).

2.4. Functional assays

2.4.1. Distortion-product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs)

DPOAEs and ABRs were used to monitor hearing loss. For testing, mice were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (13 mg/kg). Body temperature was monitored using a rectal probe and maintained at $37.5 \pm 1.0^\circ\text{C}$ using an isothermal pad. DPOAEs were measured using TDT System III hardware and EMAV software (Tang et al., 2015). DPOAEs were elicited by two pure tones, F1 and F2, with an F2/F1 ratio of 1.2. The level of F1 was set at 75 dB SPL and F2 at 65 dB SPL. F2 frequency was swept from 5 to 40 kHz in 1/8 octave steps. One hundred sweeps were presented at each test frequency. A modified Knowles miniature microphone (FG-23329-P07) and custom pre-amp were used to record responses, which were then displayed and stored on a laboratory computer.

For ABR assays, thresholds were obtained by presenting tone bursts at 5, 10, 20, 28.3, and 40 kHz from 90 dB SPL descending to 0 dB SPL or 10 dB below threshold. Tones were 5 ms in duration, 0.5 ms rise/fall, with a repetition rate of 20/second. Electrodes were placed subdermally behind the tested ear (reference), the vertex (active), and mid-back (ground). The contralateral pinna was compressed with a spring-loaded clip to reduce contribution from the unstimulated ear. Evoked potentials were collected using a Tucker Davis Technology (TDT) RZ6 processor and BioSigRZ software. Evoked potentials were averaged over 512 repetitions. ABR thresholds were obtained for the protected ear (blocked during noise exposure) before and 2 weeks after noise trauma. For the

salicylate model, ABR thresholds were determined 1 h after the injection.

2.4.2. Optical imaging of neural activity in the inferior colliculus

The voltage-sensitive dye (VSD) NK3630 (Nippon Kankoh-Shikiso Kenkyusho Co., Ltd., Japan) was used to record activity in brain slices of the central nucleus of the inferior colliculus (CNIC) in mice 4 months after noise exposure. Methods for obtaining VSD recordings in CNIC slices were previously described (Chandrasekaran et al., 2013; Li et al., 2014), and are briefly outlined here. Animals were anesthetized with isoflurane and decapitated. A block containing the inferior colliculi (IC) was removed from the brain with two transverse cuts, glued to the cutting stage of a vibratome (Dosaka, Japan), and cut into 150 μm -thick transverse slices. Slices were stored and recorded at 35 °C in oxygenated (95% O₂/5% CO₂) artificial cerebrospinal fluid (ACSF) (in mM: 120 NaCl, 3 KCl, 2 CaCl₂, 1.3 MgSO₄, 1 NaH₂PO₄, 20 NaHCO₃, 25 glucose, pH 7).

Slices were incorporated with NK3630 dissolved in ACSF at a concentration of 5–10 $\mu\text{g}/\text{ml}$ for 2–3 h, washed, and then transferred to a temperature-controlled recording chamber at 35 °C on an upright Olympus microscope (BX51W). Slices were transilluminated by light emitted from a 100W tungsten-halogen bulb passing through a 705 nm (BW 15 nm; Chroma) filter, and images were collected with a 464-element photodiode array (WuTech Instruments, Gaithersburg, MD) through a 5 x (dry, NA 0.1) objective lens at 1600 frames/second. A digital camera fitted on a second port was used to photograph the slice, on which VSD images were superimposed.

2.5. Data analysis

All analyses for the behavioral data were conducted with the Statistical Package for the Social Sciences (SPSS), Version 23.0 (SPSS Inc., Chicago, IL, USA Chicago, IL). A chi-squared (χ^2) and one-sided Fisher's exact test was applied to the Go and No-Go correct rate for individual animals. The No-Go correct rate was analyzed by χ^2 test to determine if the animal developed tinnitus-like behavior. Repeated measures within a generalized linear model were used to analyze Go correct rate and No-Go error rate. Within-group variability was analyzed through Mauchly's test of sphericity. Post hoc analyses of main effects were conducted with Fisher's least significant difference test (LSD). Significance was assumed at a *p*-value of 0.05 in all statistical analyses.

For VSD analysis, data were collected and analyzed using NeuroPlex software (RedShirtImaging, Fairfield, CT) and displayed as traces for numerical analysis and pseudocolor images for still time-lapse and real-time activity patterns. The resting light intensity was subtracted from all data. Recordings of spontaneous activity were collected for durations of 10–15 s. Portions of these responses were illustrated in traces accompanying the optical images. Random occurrence of spontaneous activity in the slices was corrected for by collecting spontaneous recordings several times and analyzing data by freezing activity in 2 s intervals and counting the number of pixels with activity above noise (S/N > 5). Thus, the same pixel could be deemed active at different times during the recording. Response activity threshold was set at 5% above baseline noise for pixel activation within each slice. Slices with less than 5% response above baseline were said to completely lack activity. To illustrate spontaneous patterns, we selected pixels in which the activity was representative of the predominant pattern.

Spontaneous bursts were observed as regions of rapid spike-like events. An activity pattern was labeled a burst if it consisted of periods of high-frequency firing separated from each other by at least 1 s (Li et al., 2014). Burst durations varied between 2 and 5 s. Each bursting period contained at least 10 spike-like events, with the duration of each spike <0.05 s. A single spike in a 2 s period was

not considered a burst. Oscillatory behavior generally consisted of slow events with clear positive and negative deflections. The duration of each oscillatory event was >0.5 s. Regions of oscillations did not contain either single spikes or rapid bursting patterns. These slow optical events have been previously shown to arise from the recruitment of large regions of the IC into synchronous firing that alternates between slow excitation and inhibition (Chandrasekaran et al., 2013). This slow synchronous activity was used to distinguish oscillations from the more rapid-firing pattern of bursts.

Trials were repeated several times (4–10 trials) on a single slice, with averages taken for each slice in our sample. The averaged data were further averaged across all slices. Origin software was used for data analysis. Data were first tested for distribution normality (Shapiro-Wilk), and then analyzed for significance with analysis of variance (ANOVA). Results are expressed as means \pm standard deviation. Significance was determined using Student's *t*-test or ANOVA (*p* < 0.05 with the Bonferroni correction factor applied).

For ABR threshold and DPOAE amplitude analysis, one-way or two-way ANOVA with repeated measures was performed by SPSS software 23.0, with time serving as the within-group variable, and frequency and tinnitus as the between-group variables, depending on the experimental design. Within-group variability was analyzed through Levene's test for homogeneity of variances. The Mann-Whitney *U* test with Bonferroni's correction was used as a post hoc test for multiple comparisons, while Dunnett's post hoc comparisons were used where appropriate. In all cases, a *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Salicylate-induced tinnitus behavior

C57BL/6J mice at 2 months of age (*n* = 21, 10 females/11 males) were trained to achieve a >90% correct rate for Go trials and a <10% error rate for No-Go trials. The mice were then tested for tinnitus-like behavior one hour after salicylate injection (350 mg/kg, i.p.; Fig. 2A). For both the Go and No-Go trials, animals achieved a correct rate close to 100% by the 15th day of the training period (Fig. 2B,C; Go: 100% \pm 0%; No-Go: 98.76% \pm 1.60%). The maintenance of correctness in the Go task after salicylate injection suggests that motor, motivation, learning, and memory functions were unimpaired (Fig. 2D). Following salicylate injection, mice showed a statistically significant increase in error rate for the No-Go task. An increase in the group error rate was observed beginning on the first day of testing and continued throughout the testing period (Fig. 2E). To determine whether this increase in error rate was due to the injection procedure itself, rather than the salicylate, an additional 11 mice (6 males, 5 females) went through the same training, and were subsequently tested after 3-day injections of saline (one injection/day), followed by additional testing after 3-day injections of salicylate. A comparison of pre- and post-saline injection revealed that no significant change in error rate occurred as a result of the injection itself. Furthermore, a significant increase in error rate was observed between the pre- and post-salicylate injection for the No-Go task (Fig. 2F,G; *t*-test, *p* < 0.01). Taken together, the lack of error in the Go task and increased error rate in the No-Go task indicates that SBAD was successful in identifying the presence of acute tinnitus induced by salicylate and these results were not confounded by injection itself.

3.2. Noise-induced tinnitus behavior

As with the salicylate-induction method, animals were trained on the Go/No-Go tasks prior to noise exposure. After the training period, unilateral noise exposure was followed by behavioral tests

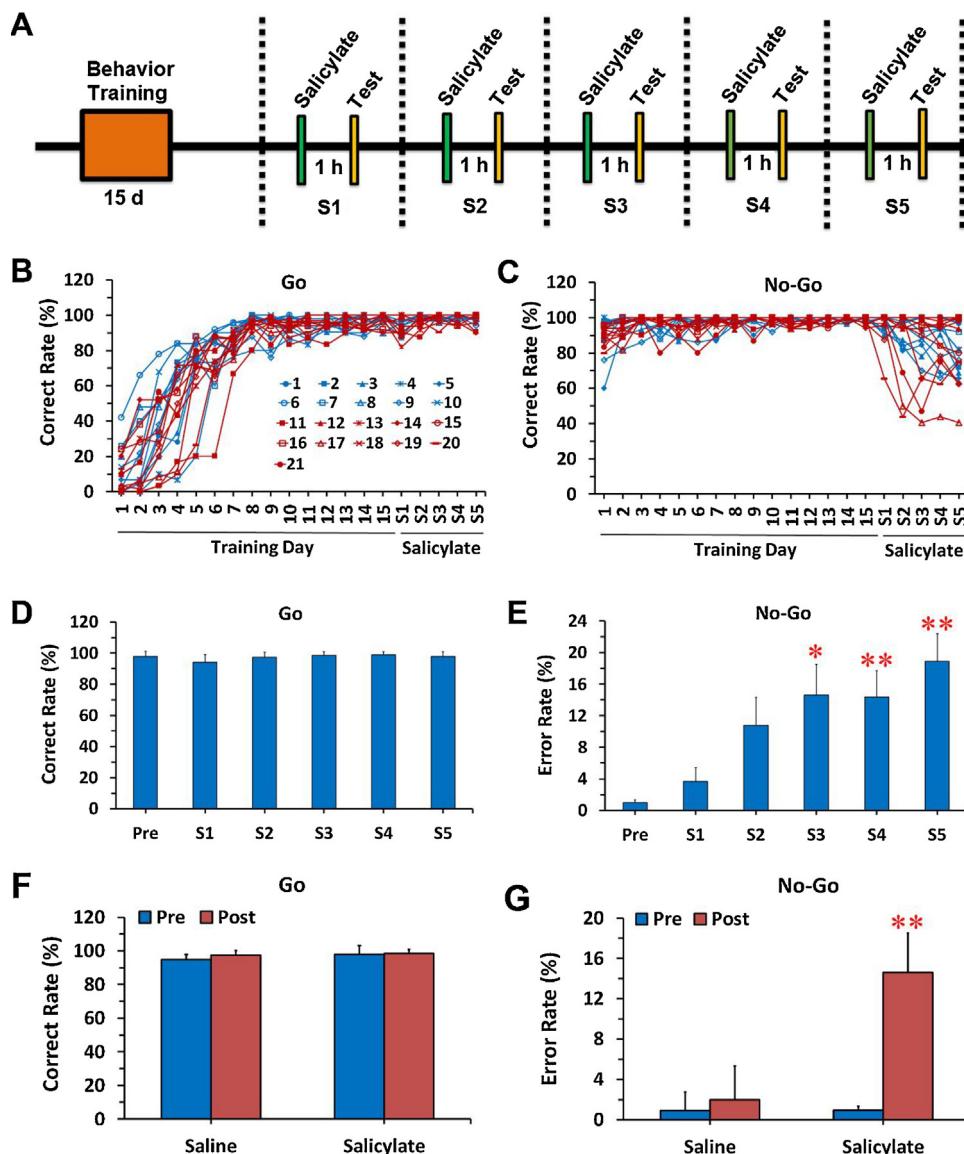


Fig. 2. SBAD results in the salicylate-induced tinnitus model.

A, The salicylate model. Behavioral training for the Go/No-Go paradigm were carried out over a period of 15 days. **B,C**, The correct rate changes of Go (B) and No-Go (C) for individual male (grey) and female (black) mice. **D**, Quantitative comparison of the Go correct rates between the control and salicylate-injected mice. Data represented by mean \pm SD, n = 21. **E**, Quantitative comparison of the No-Go correct rates between the control and salicylate-injected mice. Data represented by mean \pm SE, n = 21. Repeated measures ANOVA was used for statistical analysis. Salicylate vs Pre-salicylate, *p < 0.05, **p < 0.01. **F,G**, Quantitative comparison of both Go and No-Go tasks for mice between saline and salicylate injection. The correct rates were collected 1 day before the injections (pre) and 3 days after the injections (post). Data represented by mean \pm SE, n = 11 (6 males). Student *t*-test was used for statistical analysis. **p < 0.01.

with the Go/No-Go paradigm 2 weeks, 1 month, and 2 months after noise exposure (Fig. 3A). Go and No-Go trials were performed on 22 animals: 9 males and 13 females. For both the Go and No-Go trials, nearly all animals achieved a correct rate above 90% by the 8th day of the training period (Go: 95.65 \pm 4.96%; No-Go: 97.01 \pm 5.67%) (Fig. 3B,C). In the Go trial, noise exposure did not alter the correct rate. At 1 month and 2 months, for example, the correct rates were 96.31 \pm 2.60% and 95.58 \pm 3.88%, respectively (Fig. 3B,D). As with the salicylate model, a consistently low error rate throughout each Go trial suggests that motor, motivation, learning, and memory functions were unimpaired by unilateral noise exposure.

As opposed to Go trials, the No-Go task was accompanied by significant errors that were evident by the first testing at 2 weeks after noise exposure ($p = 0.026$), and this increase in error rate was maintained from 1 month to 2 months (Fig. 3E). There were no significant differences in error rate between the male and female groups at

2 weeks ($t = 0.480$, $p = 0.636$), 1 month ($t = 1.019$, $p = 0.320$), or 2 months ($t = 1.757$, $p = 0.093$) (Fig. 3C). Given the fact that a major advantage of the SBAD method is its ability to detect tinnitus in individual animals, we were able to use chi-square nonparametric testing to compare error rates 1 day before the noise exposure and 2 months after the exposure for each mouse. Results indicated that a significant difference was found for 11 mice (2 males and 9 females) among a total of 22 mice, suggesting the presence of tinnitus in 50% of the mice after the noise exposure. Thus, the SBAD No-Go task was successfully used to detect the presence of chronic tinnitus in individual mice.

3.3. Hearing loss from salicylate and noise

Hearing thresholds were monitored for all mice to ensure that they were able to hear the sound signal. For salicylate treatment

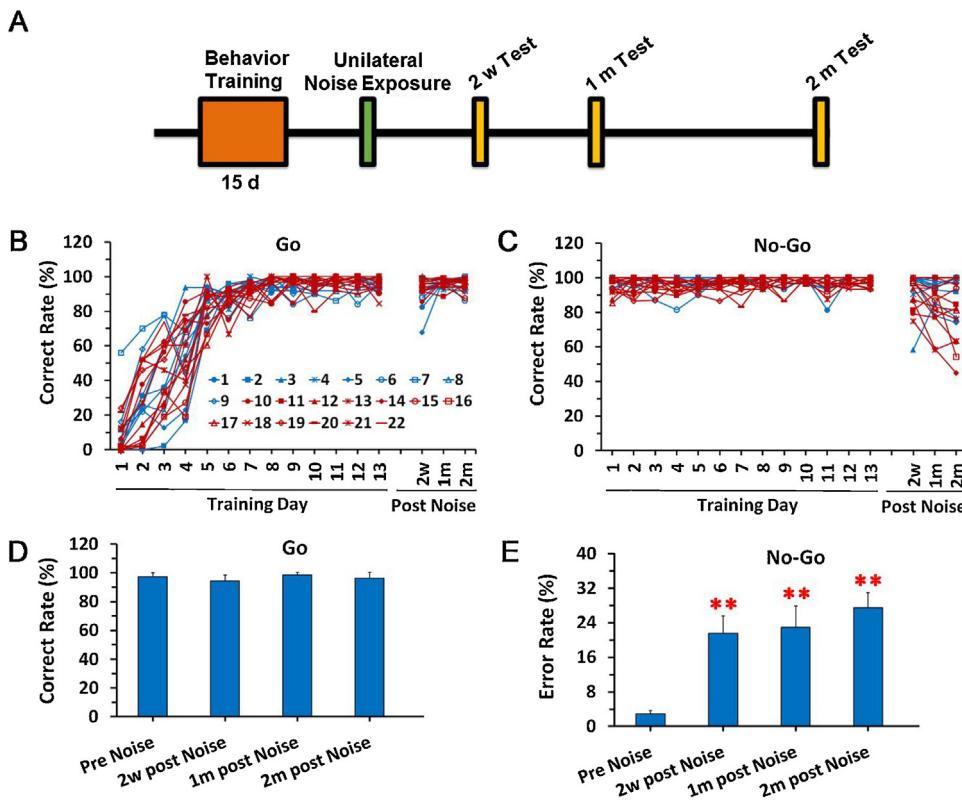


Fig. 3. SBAD results in the noise-induced tinnitus model.

A, The noise-induced model. SBAD testing was done 2 weeks after noise exposure, and then each month for 2 months. **B, C**, The correct rate changes of Go (B) and No-Go (C) for individual male (grey) and female (black) mice. **D**, Quantitative comparison of the Go correct rates after noise exposure. Data are represented by mean \pm SD ($n = 22$). **E**, Quantitative comparison of the No-Go correct rates after noise exposure. Data are represented by mean \pm SE, $n = 22$. Note: Repeated measures ANOVA was used for statistical analysis. Post-noise vs Pre-noise, ** $p < 0.01$.

(Fig. 4A), a significant increase in ABR thresholds was observed: $F(1, 25) = 379.106$, $\eta^2 = 0.938$, and $p < 0.001$. The average increase was 17.8 dB SPL one hour after the treatment. The largest change occurred at 40 kHz: from 41.6 ± 2.6 to 60.0 ± 5.5 dB SPL. Importantly, this was still well below the lowest sound intensity (75 dB SPL) used for the SBAD method. There was also a significant decrease in DPOAE amplitudes 1 h after the treatment (Fig. 4B). One-way repeated measure ANOVA indicated a functional change of outer hair cells [$F(1, 125) = 44.540$, $\eta^2 = 0.263$, and $p < 0.001$]. For the noise-induction model (Fig. 4C,D), a significant loss of hearing was observed in the unprotected ear across five frequencies tested: at 5 kHz, $F(1, 98) = 65.276$, $\eta^2 = 0.916$, $p = 0.004$; at 10 kHz, $F(1, 98) = 264.086$, $\eta^2 = 0.987$, $p < 0.001$; at 20 kHz, $F(1, 98) = 454.200$, $\eta^2 = 0.986$, $p < 0.001$; at 28.3 kHz, $F(1, 98) = 144.396$, $\eta^2 = 0.964$, $p < 0.001$; and at 40 kHz, $F(1, 98) = 99.831$, $\eta^2 = 0.968$, $p < 0.000$. However, except at 40 kHz, there were no significant changes of overall ABR thresholds in the protected (plugged) ear: $F(1, 98) = 0.482$, $\eta^2 = 0.05$; and $p = 0.489$. The average ABR increase across all five frequencies was only approximately 1 dB. Moreover, no significant change was detected in overall DPOAEs by one-way repeated measure ANOVA: $F(1, 450) = 3.184$, $\eta^2 = 0.007$, and $p = 0.075$. Therefore, the earplugs were effective and mice were still able to hear the sound signals used in the SBAD test.

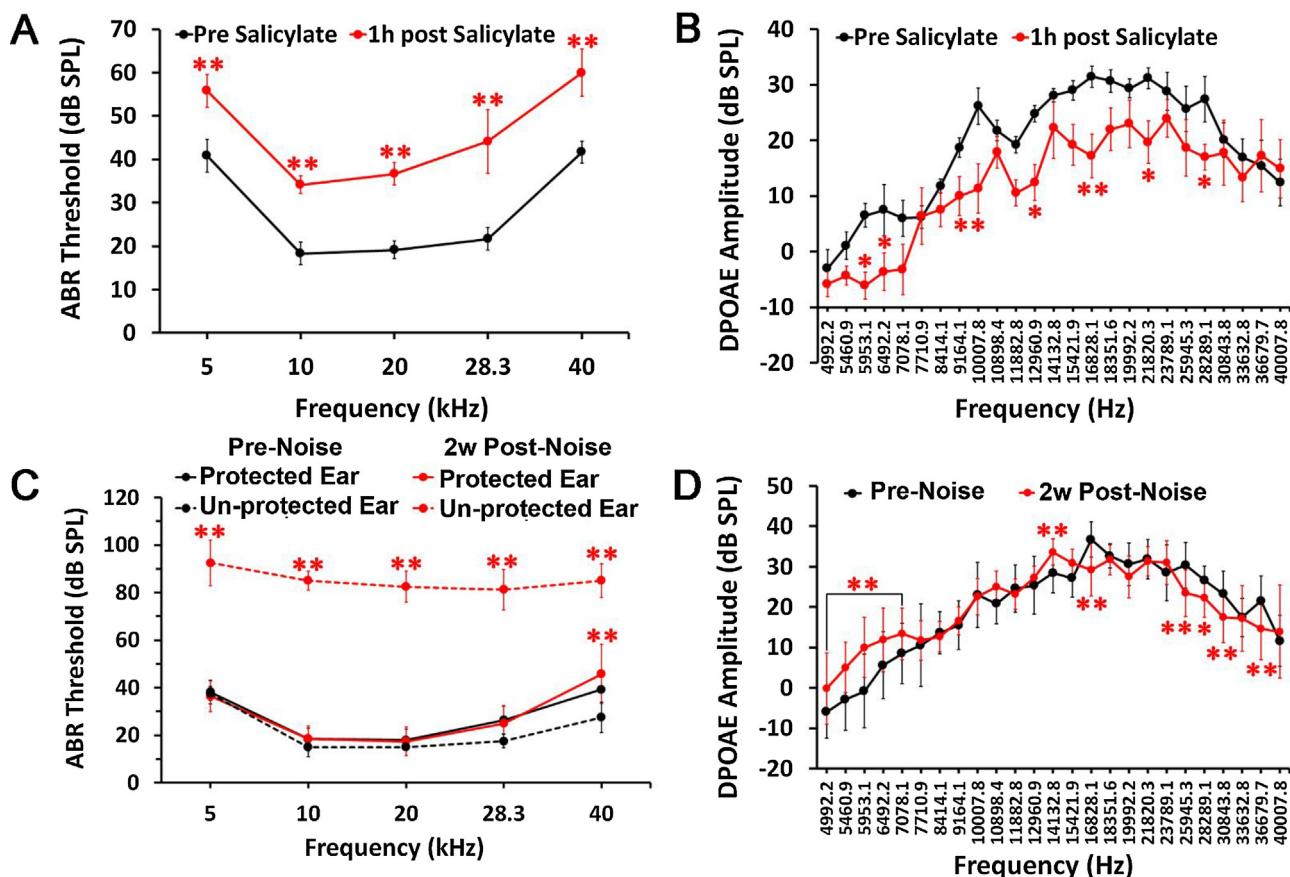
3.4. Changes of ABR wave V/I ratios

Previous human studies have clearly showed that tinnitus subjects have reduced ABR wave I amplitudes and increased wave V amplitudes (Gu et al., 2012). Furthermore, changes of ABR wave amplitude were also observed in a mouse model with salicylate-induced tinnitus (Lowe and Walton, 2015). To determine whether

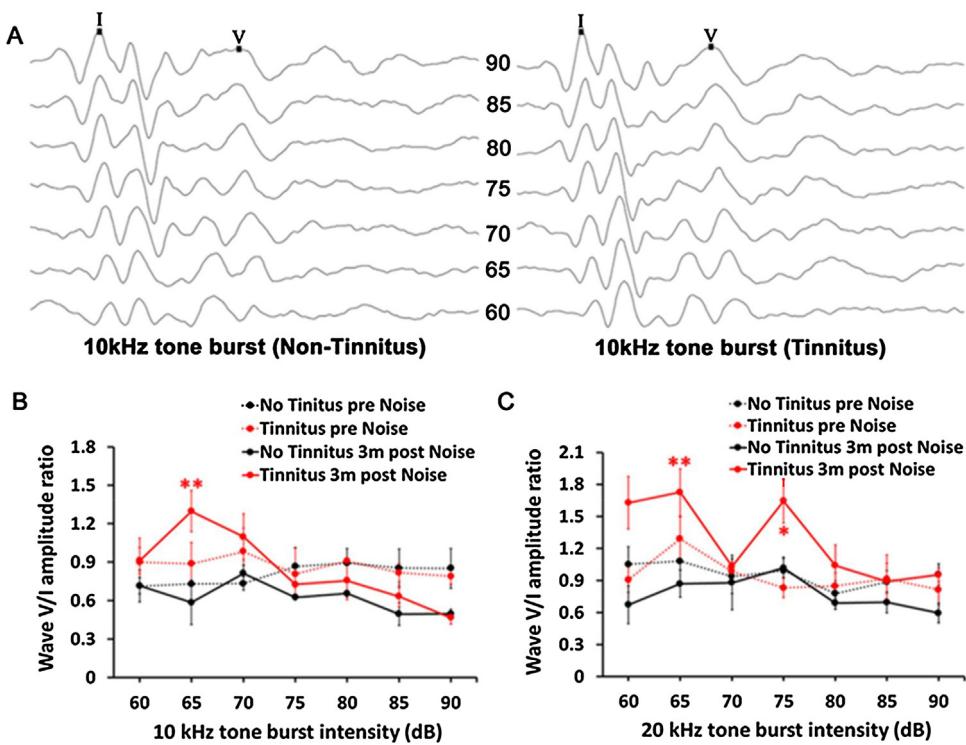
the SBAD correlates of tinnitus were accompanied by changes in neural activity, we quantified the ratios of the amplitudes of ABR waves V and I, which correspond, respectively, to the levels of neuronal activity in the IC and auditory nerve (Fig. 5). The amplitude ratios were quantified at 10 kHz and 20 kHz by measuring peak values relative to baseline. Animals were grouped according to whether they did or did not develop tinnitus following noise exposure (chi-squared test $p < 0.05$). Two-way repeated measure ANOVA was used for ABR wave V/I ratio analysis. Overall, the tinnitus group showed a significant increase of wave V/I ratio over the non-tinnitus group for both 10 kHz ($F = 9.653$, $p = 0.010$) and 20 kHz ($F = 12.886$, $p = 0.002$). The largest differences in the wave V/I ratio were observed in animals that had developed tinnitus following noise exposure. In this group of animals, the wave V/I ratio was >1 at both 10 kHz and 20 kHz. In non-tinnitus mice, ABR wave V/I ratios were not significantly different between before and after noise exposure (Fig. 5B,C). These data validate the SBAD method, and further suggest that the midbrains of mice with tinnitus exhibit increases in synchronous activity.

3.5. Spontaneous activity in the IC

To further validate the SBAD method, we used optical imaging with VSDs to determine whether the CNIC from tinnitus-positive mice detected by the SBAD method exhibited abnormal levels of spontaneous activity. A total of 20 C57BL/6J mice at 2 months old were trained and tested until 4 months old. Three representative examples were showed in Fig. 6A–C. A control mouse with no noise exposure and no tinnitus (Fig. 6A) produced a CNIC that exhibited occasional spontaneous activity. There was little synchronous firing and the low neuronal activity that we observed did not man-

**Fig. 4.** Hearing loss in the salicylate- and noise-induced tinnitus models.

A, ABR thresholds 1 h after salicylate injection ($n=6$). **B**, DPOAE amplitudes from the same animals 1 h after salicylate injection. **C**, ABR thresholds of the protected ears before and 2 weeks post-noise exposure ($n=20$). **D**, DPOAE amplitudes from the same mice. **Note:** Data (A-D) is represented by mean \pm SD, and * $p < 0.05$, ** $p < 0.01$.

**Fig. 5.** ABR wave V/I ratios after noise exposure.

Quantifications of ABR wave V/I amplitude ratios. **A**, The representative ABR wave at tone burst 10 kHz for non-tinnitus (left) and tinnitus animals (right). **B**, **C**, The quantitative analysis of ABR wave V/I peak value at tone burst 10 kHz (B) and 20 kHz (C), respectively. Data were represented by mean \pm SE; No tinnitus, $n=7$; Tinnitus, $n=5$. * $p < 0.05$, ** $p < 0.01$.

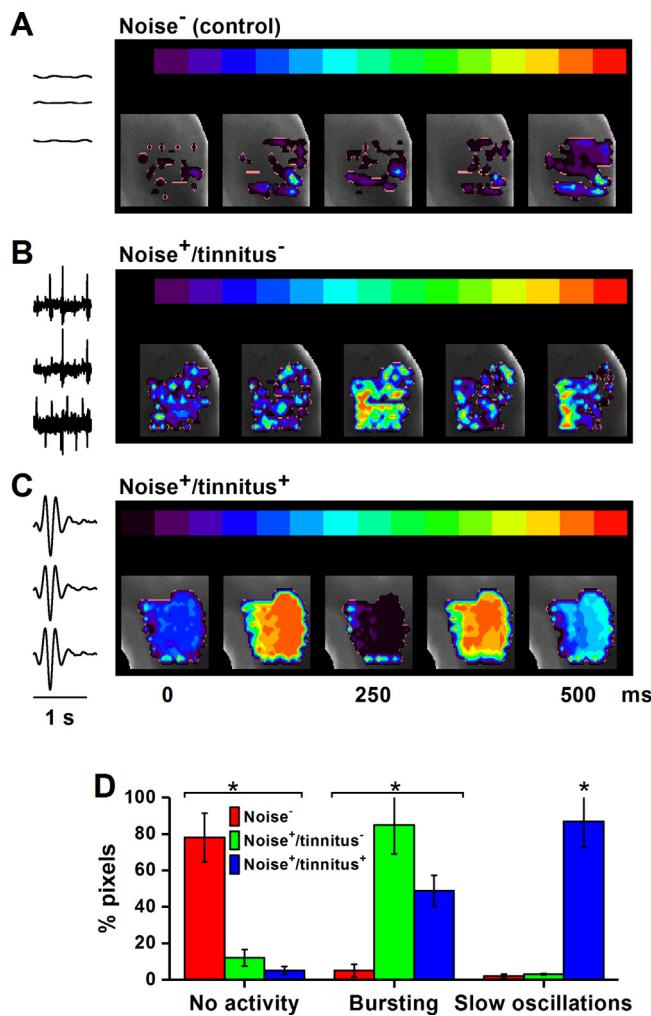


Fig. 6. Optical imaging of neuronal activity in the CNIC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Spontaneous activity in the CNIC under three conditions with recording traces on the left side and lapse images on the right side. **A**, no exposure to noise (Control); **B**, exposure to noise without development of tinnitus ($\text{Noise}^+/\text{tinnitus}^-$); **C**, exposure to noise with tinnitus development ($\text{Noise}^+/\text{tinnitus}^+$). Color scale: violet (minimum) to red (maximum). Violet through red are relative magnitudes of activity on either side of the baseline (green). Negative deflections from the baseline are depicted from a movement toward violet and positive deflections toward red. **D**, summary of spontaneous activity in the conditions indicated. Averages from 17 slices from 5 animals in each condition. Data were represented by mean \pm SD.

ifest as large-amplitude events above baseline (Fig. 6A, recording traces on the left side and lapse images on the right side). One mouse that had been exposed to noise, but was tinnitus-negative ($\text{noise}^+/\text{tinnitus}^-$), yielded CNIC slices exhibiting increased levels of spontaneous activity. Single events clearly above baseline manifested as synchronous activity in different regions of the CNIC (Fig. 6B, recording traces on the left side and lapse images on the right side). Spontaneous activity was widespread and occurred randomly within the CNIC. In CNIC slices from one mouse that had been exposed to noise and was tinnitus-positive ($\text{noise}^+/\text{tinnitus}^+$), slow oscillations were highly prevalent. In the same broad region of the CNIC, oscillatory activity switched from positive excitatory responses to inhibitory responses below baseline levels (Fig. 6C, recording traces on the left side and lapse images on the right side). Oscillatory activity in $\text{noise}^+/\text{tinnitus}^+$ animals differed from the spontaneous bursting observed from $\text{noise}^+/\text{tinnitus}^-$ animals in their capacity to elicit synchronous responses within large areas of the CNIC. In addition to exhibiting slow oscillations, the CNIC

of $\text{noise}^+/\text{tinnitus}^+$ mice also gave rise to random rapid bursting. These observations were quantified and compared for these three groups of mice (Fig. 6D). These data are consistent with previous studies showing large-scale increases in synchronous activity in the IC with tinnitus, further validating the SBAD method.

4. Discussion

These data demonstrate the ability of SBAD for detecting tinnitus in mice. In the salicylate-induced tinnitus model, the SBAD method was able to detect a significant increase in error rate during No-Go trials. In the noise-induction model, the SBAD method consistently indicated tinnitus-like behavior for at least two months following noise exposure. Together, these data suggest that the SBAD method can be used to monitor both acute and chronic tinnitus. Although this method is not faster than the GPIAS method, it is less time-consuming than most existing operant-based methods for the detection of tinnitus in mice. What's more, despite major differences in the neural pathways involved in salicylate- versus noise-induced tinnitus, (e.g., Chambers et al., 2016; Kaltenbach, 2000; Sun et al., 2009), the ability of the SBAD method to detect tinnitus in both models indicates that it may be a useful tool to study tinnitus, irrespective of the neuronal pathway activated.

4.1. Tinnitus induction

Understanding the underlying pathologies of tinnitus-induction models can assist us in choosing the most appropriate method for developing and validating tinnitus-detection tests. The two common methods for inducing tinnitus are pharmacological and acoustic manipulations. The pharmacological method most commonly used by tinnitus researchers is salicylate induction because salicylate has been well-studied in animals (for review, Stolzberg et al., 2012). Salicylate interacts with the auditory system at multiple levels. In the cochlea, it acts as a competitive antagonist for the chloride anion binding site of prestin in the outer hair cells (Oliver et al., 2001) and subsequently changes hearing sensitivity, which may play a role in determining the pitch of tinnitus (Stolzberg et al., 2011). In the brain, salicylate downregulates serotonin (Caperton and Thompson, 2011) and γ -aminobutyric acid (GABA) activity (Bauer et al., 2000), and also affects the conductivity of potassium channels (Stolzberg et al., 2012). Interestingly, whereas noise-induced tinnitus has been found to stimulate the lemniscal auditory pathway (the primary pathway by which tonotopic sound information travels from the cochlea through the brainstem to auditory parts of the brain), acute systemic salicylate treatment causes hyperactivity in the extralemniscal pathway (the non-primary auditory pathway by which non-tonotopic sound information travels to the brain) (Eggermont and Kenmochi, 1998). Thus, past studies demonstrate heterogeneity in different central mechanisms between salicylate-induced and noise-induced tinnitus methods (Roberts et al., 2010). Two main advantages of salicylate-induced tinnitus are its relatively fast induction and its reversibility. The acute tinnitus phenotype was ideal for validation of the initial testing design. Although this method can reliably induce tinnitus in laboratory animals, its main disadvantage is its minimal relevance to the common human chronic tinnitus pathology.

Acoustic trauma is the most popular method for inducing chronic tinnitus. One of the most consistent features among many studies is unilateral noise exposure, mainly because either GPIAS or OC-based tests will require at least one functioning ear. Acoustic trauma induces a number of acute and chronic changes in the cochlea and central nervous system. At the periphery, acoustic trauma can result in synapse loss, loss of hair cells, and subsequent

death of spiral ganglion neurons (e.g., Wang et al., 2002; Kujawa and Liberman, 2009). The changes after acoustic trauma at the different levels of the ascending auditory pathways are manifold and complex. Within hours after acoustic trauma, the spontaneous neuronal activity in the primary auditory cortex (A1) of the cat increases in the frequency region below the damage (Noreña and Eggermont, 2005). Weeks after an acoustic trauma, the tonotopic map of A1 reorganizes so that there are no neurons with characteristic frequencies above the frequency of the traumatizing stimulus (Noreña and Eggermont, 2005). Neurons in the inferior colliculus exhibit increased spontaneous firing rates after the trauma (Bauer et al., 2008). Changes in the dorsal cochlear nucleus, similar to the cochlea, may only contribute to the tinnitus initiation but not to the final perception of tinnitus (Bauer and Brozoski, 2001). An increase in spontaneous activity has been observed after the trauma, specifically in fusiform cells (e.g., Kaltenbach et al., 2002, 2004; Finlayson and Kaltenbach, 2009). Recently, intricate contributions of ion channels in early tinnitus induction due to acoustic trauma have been elucidated in the dorsal cochlear nucleus (Li et al., 2013, 2015). The main advantage of this tinnitus induction is that it is highly relevant to human pathology. Its major disadvantage is that this method often leads to variable percentages of animals showing tinnitus-like behavior (e.g., Chen et al., 2013), which was also observed in this study.

4.2. Validation of the SBAD method

Because animals such as mice cannot directly report their tinnitus status, behavior screening remains a contentious issue for animal studies of tinnitus (Jones and May, 2016). Here, the detection outcome of the SBAD method was validated by two functional assays. ABR wave I is believed to originate from the cochlear hair cells and auditory nerve, and ABR wave V from the IC. A recent ABR study of human patients with tinnitus found that the wave V/I amplitude ratios were elevated when compared to matched patients without tinnitus (Gu et al., 2012), and this finding was subsequently supported by animal studies (Lowe and Walton, 2015). Based on these findings, we quantified the wave V/I amplitude ratios, and found that mice with tinnitus detected by the SBAD method showed an increased V/I amplitude ratio, strongly validating the SBAD method. In addition, the significant difference was unexpectedly found at low intensities (65 and 75 dB SPL), suggesting possible involvement of large afferents from spiral ganglion neurons in tinnitus generation. Because the IC has long been implicated in tinnitus pathology (e.g., Berger and Coomber, 2015; Henry et al., 2014), we examined gross neuronal activity in the CNIC. The outcome was also consistent: there is a high degree of neuronal activity in mice with tinnitus detected by the SBAD method, again validating the SBAD's ability to detect tinnitus.

4.3. Comparison of the SBAD method with the GPIAS method

The GPIAS method is now widely used for detecting tinnitus in mice, mainly because of its elegant concept and straightforward procedures (e.g., Dehmel et al., 2012; Lowe and Walton, 2015; Middleton et al., 2011; Yang et al., 2007). Despite its advantages, numerous refinements have been required to address high variability in results generated when using the GPIAS method (Lobarinas et al., 2013; Longenecker and Galazyuk, 2011, 2012). While these refinements have reduced the chance of false outcomes, the GPIAS relies on the assumption that subjects with tinnitus cannot perceive silence when it is presented in a brief gap of time. The validity of this assumption has come into question in recent years and doubt has been cast in terms of the extent to which sound perception in tinnitus "fills" silent gaps. In human studies, patients reporting tinnitus (mostly above 500 Hz) showed a GPIAS deficit at both 500

and 4000 Hz (Fournier and Hebert, 2013). Given that human subjects in the study were not well matched, the difference between control and tinnitus subjects could be due to other factors such as age, gender, and hearing thresholds (particularly high-frequency hearing loss). Two subsequent studies directly tested the "filling-in" assumption and found no frequency-specific gap-detection deficit in patients with tinnitus (Campolo et al., 2013; Boyen et al., 2015). Similar inconsistency has been reported in animal studies (Hickox and Liberman, 2014; Radziwon et al., 2015). In the SBAD method, although we make a similar assumption that mice with tinnitus are unable to perceive silence, we do not assume that mice with tinnitus will be aware of the perception of a "phantom sound" and we also don't attempt to match sound stimuli to a particular frequency. We train mice to respond to several different sound stimuli by crossing quickly to the opposite side of a shuttle box. With this training, we expect that animals with tinnitus would cross to the other side during both Go and No-Go tasks because they perceive sound even during the No-Go trials.

Acoustic trauma may bring its own complexities. Because GPIAS relies on a ratio, any change in the ratio can reflect changes in the numerator or denominator. In fact, noise injury leads to an enhanced startle reflex that is most likely due to hyperacusis—an increased sensitivity to external sounds—rather than tinnitus (Hickox and Liberman, 2014). Beyond these issues are problematic claims that the tinnitus pitch can be identified by matching it to the pitch of the background noise. This claim requires the consideration of all pitch and level cues a listener might use to detect one stimulus that is masked by another. Recent data in rats suggest that this interpretation is not always straightforward (Jones and May, 2016). With the establishment of our SBAD method, it could cross-validate GPIAS and SBAD methods in the same mouse.

4.4. Potential limitations and future modifications of the SBAD method

Although the current SBAD method has proven itself capable of detecting tinnitus in mice, there are three main limitations. First, like almost all current behavioral methods to detect tinnitus, the SBAD method relies on a presumed inability of animals to distinguish their internal tinnitus from an external sound. This is not demonstrably true in humans, and it is not known whether it could be true in all animals. One recent method in rats eliminates this assumption by using stimulus classification and tinnitus generalization approaches (Jones and May, 2016). Similar modifications to the SBAD procedure will eliminate this limitation and allow our method to indicate tinnitus pitch, an important clinical tinnitus parameter. Second, the current SBAD method has only been tested in C57BL/6J mice. Other inbred strains should be tested in future studies. In addition, it has been proposed that mammals that do not hear below 0.5 kHz do not use temporal encoding for pitch perception (Heffner et al., 2001). Therefore, the SBAD method should be tested in other animal models with a hearing frequency range similar to humans. Third, an extinction response is commonly observed in the absence of reinforcement in most OC methods, and false-positive errors could result if the trained responses of No-Go trials were extinguished with repeated testing. Because active avoidance learning is less likely to result in extinction behavior, we adopted it for the SBAD method. We also applied two innovations to reduce the possibility of extinction (see Materials and Methods). The chance of extinction could still exist with extensive repeated measurement. A reduction of repeated testing sessions with a modified experimental design may help to diminish this possibility.

5. Conclusions

This study addresses an urgent need in the field of tinnitus research and provides a new OC-based method that effectively detects tinnitus-like behavior in mice. Its detection ability is further validated by data from functional studies such as the abnormal increase of neuronal activity in the cochlea and middle brain of mice identified as having tinnitus by the SBAD method. Major advantages of this adapted approach are: (1) this method utilizes a “Go” paradigm to monitor possible confounding factors such as learning and motivation; (2) this method can detect tinnitus in individual animals in comparison to some operant methods, which could only detect group differences; and (3) this method can be repeated to detect chronic tinnitus. Thus, this new method will lead to a deeper understanding of the molecular pathways underlying tinnitus pathology. In addition, the affordability of mouse models could further aid in the development of more efficient drug screening trials aimed at preventing and treating tinnitus.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jneumeth.2017.08.029>.

References

- Bauer, C.A., Brozoski, T.J., 2001. Assessing tinnitus and prospective tinnitus therapeutics using a psychophysical animal model. *J. Assoc. Res. Otolaryngol.* 2, 54–64.
- Bauer, C.A., Brozoski, T.J., Holder, T., Caspary, D.M., 2000. Effects of chronic salicylate on GABAergic activity in rat inferior colliculus. *Hear. Res.* 147, 175–182.
- Bauer, C.A., Turner, J.G., Caspary, D.M., Myers, K.S., Brozoski, T.J., 2008. Tinnitus and inferior colliculus activity in chinchillas related to three distinct patterns of cochlear trauma. *J. Neurosci. Res.* 86, 2564–2578.
- Berger, J.I., Coomber, B., 2015. Tinnitus-related changes in the inferior colliculus. *Front. Neurol.* 6, 61.
- Boyen, K., Başkent, D., van Dijk, P., 2015. The gap detection test: can it be used to diagnose tinnitus? *Ear Hear.* 36, e138–e145.
- Brozoski, T.J., Bauer, C.A., 2016. Animal models of tinnitus. *Hear. Res.* 338, 88–97.
- Campolo, J., Lobatinas, E., Salvi, R., 2013. Does tinnitus fill in the silent gaps? *Noise Health* 15, 398–405.
- Chambers, A.R., Resnik, J., Yuan, Y., Whitton, J.P., Edge, A.S., Liberman, M.C., Polley, D.B., 2016. Central gain restores auditory processing following near-Complete cochlear denervation. *Neuron* 89, 867–896.
- Chandrasekaran, L., Xiao, Y., Sivaramakrishnan, S., 2013. Functional architecture of the inferior colliculus revealed with voltage-sensitive dyes. *Front. Neural. Circ.* 7, 41.
- Chen, G., Lee, C., Sandridge, S., Butler, H., Manzoor, N., Kaltenbach, J., 2013. Behavioral evidence for possible simultaneous induction of hyperacusis and tinnitus following intense sound exposure. *J. Assoc. Res. Otolaryngol.* 14, 413–424.
- Crocetti, A., Forti, S., Ambrosetti, U., Bo, L.D., 2009. Questionnaires to evaluate anxiety and depressive levels in tinnitus patients. *Otolaryngol. Head Neck Surg.* 140, 403–405.
- Dehmel, S., Eisinger, D., Shore, S.E., 2012. Gap prepulse inhibition and auditory brainstem-evoked potentials as objective measures for tinnitus in guinea pigs. *Front. Syst. Neurosci.* 6, 42.
- Estes, W.K., Skinner, B.F., 1941. Some quantitative properties of anxiety. *J. Exp. Psychol.* 29, 390–400.
- Finlayson, P.G., Kaltenbach, J.A., 2009. Alterations in the spontaneous discharge patterns of single units in the dorsal cochlear nucleus following intense sound exposure. *Hear. Res.* 256, 104–117.
- Folmer, R.L., Griest, S.E., 2000. Tinnitus and insomnia. *Am. J. Otolaryngol.* 21, 287–293.
- Fournier, P., Hebert, S., 2013. Gap detection deficits in humans with tinnitus as assessed with the acoustic startle paradigm: does tinnitus fill in the gap? *Hear. Res.* 295, 16–23.
- Grimsley, C.A., Longenecker, R.J., Rosen, M.J., Young, J.W., Grimsley, J.M., Galazyuk, A.V., 2015. An improved approach to separating startle data from noise. *J. Neurosci. Methods* 253, 206–217.
- Gu, J.W., Herrmann, B.S., Levine, R.A., Melcher, J.R., 2012. Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus. *J. Assoc. Res. Otolaryngol.* 13, 819–833.
- Hébert, S., Canlon, B., Hasson, D., Magnusson, L.L., Westerlund, H., Theorell, T., 2012. Tinnitus severity is reduced with reduction of depressive mood—a prospective population study in Sweden. *PLoS ONE* 7, e37733.
- Hayes, S.H., Radziwon, K.E., Stolzberg, D.J., Salvi, R.J., 2014. Behavioral models of tinnitus and hyperacusis in animals. *Front. Neurol.* 5, 179.
- Heeringa, A.N., Agterberg, M.J., van Dijk, P., 2014. Spontaneous behavior in noise and silence: a possible new measure to assess tinnitus in Guinea pigs. *Front. Neurol.* 5, 207.
- Heffner, H.E., Harrington, I.A., 2002. Tinnitus in hamsters following exposure to intense sound. *Hear. Res.* 170, 83–95.
- Heffner, R.S., Koay, G., Heffner, H.E., 2001. Audiograms of five species of rodents: implications for the evolution of hearing and the perception of pitch. *Hear. Res.* 157, 138–152.
- Henry, K.R., Chole, R.A., 1980. Genotypic differences in behavioral, physiological and anatomical expressions of age-related hearing loss in the laboratory mouse. *Audiology* 19, 369–383.
- Henry, J.A., Roberts, L.E., Caspary, D.M., Theodoroff, S.M., Salvi, R.J., 2014. Underlying mechanisms of tinnitus: review and clinical implications. *J. Am. Acad. Audiol.* 25, 5–22.
- Hickox, A.E., Liberman, M.C., 2014. Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? *J. Neurophysiol.* 111, 552–564.
- Jastreboff, P.J., Brennan, J.F., Sasaki, C.T., 1988a. An animal model for tinnitus. *Laryngoscope* 98, 280–286.
- Jastreboff, P.J., Brennan, J.F., Coleman, J.K., Sasaki, C.T., 1988b. Phantom auditory sensation in rats: an animal model for tinnitus. *Behav. Neurosci.* 102, 811–822.
- Jones, A., May, B.J., 2016. Improving the reliability of tinnitus screening in laboratory animals. *J. Assoc. Res. Otolaryngol.* 18, 183–195.
- Kaltenbach, J.A., Rachel, J.D., Mathog, T.A., Zhang, J., Falzarano, P.R., Lewandowski, M., 2002. Cisplatin-induced hyperactivity in the dorsal cochlear nucleus and its relation to outer hair cell loss: relevance for tinnitus. *J. Neurophysiol.* 88, 699–714.
- Kaltenbach, J.A., Zacharek, M.A., Zhang, J., Frederick, S., 2004. Activity in the dorsal cochlear nucleus of hamsters previously tested for tinnitus following intense tone exposure. *Neurosci. Lett.* 355, 121–125.
- Kaltenbach, J.A., 2000. Neurophysiologic mechanisms of tinnitus. *J. Am. Acad. Audiol.* 11, 125–137.
- Klink, K.B., Bendig, G., Klump, G.M., 2006. Operant methods for mouse psychoacoustics. *Behav. Res. Methods* 38, 1–7.
- Kujawa, S.G., Liberman, M.C., 2009. Adding insult to injury: cochlear nerve degeneration after temporary noise-induced hearing loss. *J. Neurosci.* 29, 14077–14085.
- Li, S., Choi, V., Tzounopoulos, T., 2013. Pathogenic plasticity of Kv7.2/3 channel activity is essential for the induction of tinnitus. *Proc. Natl. Acad. Sci. U. S. A.* 110, 9980–9985.
- Li, Y., Davey, R.A., Sivaramakrishnan, S., Lynch, W.P., 2014. Postinhibitory rebound neurons and networks are disrupted in retrovirus-induced spongiform neurodegeneration. *J. Neurophysiol.* 112, 683–704.
- Li, S., Kalappa, B.I., Tzounopoulos, T., 2015. Noise-induced plasticity of KCNQ2/3 and HCN channels underlies vulnerability and resilience to tinnitus. *Elife* 27, 4.
- Lobarinas, E., Sun, W., Cushing, R., Salvi, R., 2004. A novel behavioral paradigm for assessing tinnitus using schedule-induced polydipsia avoidance conditioning (SIP-AC). *Hear. Res.* 190, 109–114.
- Lobarinas, E., Sun, W., Stolzberg, D., Lu, J., Salvi, R., 2008. Human brain imaging of tinnitus and animal models. *Semin. Hear.* 29 (4), 333–349.
- Lobarinas, E., Hayes, S.H., Allman, B.L., 2013. The gap-startle paradigm for tinnitus screening in animal models: limitations and optimization. *Hear. Res.* 295, 150–160.
- Longenecker, R.J., Galazyuk, A.V., 2011. Development of tinnitus in CBA/CaJ mice following sound exposure. *J. Assoc. Res. Otolaryngol.* 12, 647–658.
- Longenecker, R.J., Galazyuk, A.V., 2012. Methodological optimization of tinnitus assessment using prepulse inhibition of the acoustic startle reflex. *Brain Res.* 1485, 54–62.
- Longenecker, R.J., Chonko, K.T., Maricich, S.M., Galazyuk, A.V., 2014. Age effects on tinnitus and hearing loss in CBA/CaJ mice following sound exposure. *Springerplus* 3, 542.
- Lowe, A.S., Walton, J.P., 2015. Alterations in peripheral and central components of the auditory brainstem response: a neural assay of tinnitus. *PLoS One* 10, e0117228.
- Middleton, J.W., Kiritani, T., Pedersen, C., Turner, J.G., Shepherd, G.M., Tzounopoulos, T., 2011. Mice with behavioral evidence of tinnitus exhibit dorsal cochlear nucleus hyperactivity because of decreased GABAergic inhibition. *Proc. Natl. Acad. Sci. U. S. A.* 108, 7601–7606.
- Noreña, A.J., Eggermont, J.J., 2005. Enriched acoustic environment after noise trauma reduces hearing loss and prevents cortical map reorganization. *J. Neurosci.* 25, 699–705.

- Oliver, D., He, D.Z., Klöcker, N., Ludwig, J., Schulte, U., Waldegg, S., Ruppertsberg, J.P., Dallos, P., Fakler, B., 2001. **Intracellular anions as the voltage sensor of prestin, the outer hair cell motor protein.** *Science* 292, 2340–2343.
- Pace, E., Luo, H., Bobian, M., Panekkad, A., Zhang, X., Zhang, H., Zhang, J., 2016. **A conditioned behavioral paradigm for assessing onset and lasting tinnitus in rats.** *PLoS One* 11, e0166346.
- Prosen, C.A., Dore, D.J., May, B.J., 2003. **The functional age of hearing loss in a mouse model of presbycusis I. Behavioral assessments.** *Hear. Res.* 183, 44–56.
- Radziwon, K.E., Stolzberg, D.J., Urban, M.E., Bowler, R.A., Salvi, R.J., 2015. **Salicylate-induced hearing loss and gap detection deficits in rats.** *Front. Neurol.* 6, 31.
- Ruttiger, L., Ciuffani, J., Zenner, H.P., Knipper, M., 2003. **A behavioral paradigm to judge acute sodium salicylate-induced sound experience in rats: a new approach for an animal model on tinnitus.** *Hear. Res.* 180, 39–50.
- Ryan, D., Bauer, C.A., 2016. **Neuroscience of tinnitus.** *Neuroimaging Clin. N. Am.* 26, 187–196.
- Schleidt, W.M., Kickert-Magg, M., 1979. **Hearing thresholds of albino house mouse between 1 and 80 kHz by shuttle box training.** *J. Audit. Res.* 19, 37–40.
- Sederholm, F., Swedberg, M.D., 2013. **Establishment of auditory discrimination and detection of tinnitus induced by salicylic acid and intense tone exposure in the rat.** *Brain Res.* 1510, 48–62.
- Shargorodsky, J., Curhan, G.C., Farwell, W.R., 2010. **Prevalence and characteristics of tinnitus among US adults.** *Am. J. Med.* 123, 711–718.
- Stolzberg, D., Chen, G.D., Allman, B.L., Salvi, R.J., 2011. **Salicylate-induced peripheral auditory changes and tonotopic reorganization of auditory cortex.** *Neurosci* 180, 157–164.
- Stolzberg, D., Salvi, R.J., Allman, B.L., 2012. **Salicylate toxicity model of tinnitus.** *Front. Syst. Neurosci.* 6, 28.
- Stolzberg, D., Hayes, S.H., Kashanian, N., Radziwon, K., Salvi, R.J., Allman, B.L., 2013. **A novel behavioral assay for the assessment of acute tinnitus in rats optimized for simultaneous recording of oscillatory neural activity.** *J. Neurosci. Methods* 219, 224–232.
- Sun, W., Lu, J., Stolzberg, D., Gray, L., Deng, A., Lobarinas, E., Salvi, R.J., 2009. **Salicylate increases the gain of the central auditory system.** *Neuroscience* 159, 325–334.
- Tang, J., Qian, Y., Li, H., Kopecky, B.J., Ding, D., Ou, H.C., DeCook, R., Chen, X., Sun, Z., Kobel, M., Bao, J., 2015. **Canertinib induces ototoxicity in three preclinical models.** *Hear. Res.* 328, 59–66.
- Tunkel, D.E., Bauer, C.A., Sun, G.H., et al., 2014. **Clinical practice guideline: tinnitus executive summary.** *Otolaryngol. Head Neck Surg.* 151, 533–541.
- Turner, J.G., Brozoski, T.J., Bauer, C.A., Parrish, J.L., Myers, K., Hughes, L.F., Caspary, D.M., 2006. **Gap detection deficits in rats with tinnitus: a potential novel screening tool.** *Behav. Neurosci.* 120, 188–195.
- Turner, J., Larsen, D., Hughes, L., Moehars, D., Shore, S., 2012. **Time course of tinnitus development following noise exposure in mice.** *J. Neurosci. Res.* 90, 1480–1488.
- Veteran Benefits Administration, 2013. **VA Research on Hearing Loss (Accessed February 13 2017)** Available at: <http://www.research.va.gov/topics/hearing.cfm>.
- Wang, Y., Hirose, K., Liberman, M.C., 2002. **Dynamics of noise-induced cellular injury and repair in the mouse cochlea.** *J. Assoc. Res. Otolaryngol.* 3, 248–268.
- Yang, G., Lobarinas, E., Zhang, L., Turner, J., Stolzberg, D., Salvi, R., Sun, W., 2007. **Salicylate induced tinnitus: behavioral measures and neural activity in auditory cortex of awake rats.** *Hear. Res.* 226, 244–253.
- Yang, S., Weiner, B.D., Zhang, L.S., Cho, S.J., Bao, S., 2011. **Homeostatic plasticity drives tinnitus perception in an animal model.** *Proc. Natl. Acad. Sci. U. S. A.* 108, 14974–14979.
- Zhang, C., Flowers, E., Li, J.X., Wang, Q., Sun, W., 2014. **Loudness perception affected by high doses of salicylate? a behavioral model of hyperacusis.** *Behav. Brain Res.* 271, 16–22.
- Zheng, Y., Stiles, L., Hamilton, E., Smith, P.F., Darlington, C.L., 2010. **The effects of the synthetic cannabinoid receptor agonists, WIN55, 212-2 and CP55 940, on salicylate-induced tinnitus in rats.** *Hear. Res.* 268, 145–150.